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# The Maternal Genes *apx-1* and *glp-1* and Establishment of Dorsal-Ventral Polarity in the Early C. elegans Embryo

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## Summary

**The sister blastomeres ABp and ABa are equipotent at the beginning of the 4-cell stage in C. elegans embryos, but soon become committed to different fates. We show that the *glp-1* gene, a homolog of the *Notch* gene of Drosophila, functions in two distinct cell-cell interactions that specify the ABp and ABa fates. These interactions both require maternal expression of *glp-1*. We show that a second maternal gene, *apx-1*, functions with *glp-1* only in the specification of the ABp fate and that *apx-1* can encode a protein homologous to the Delta protein of Drosophila. Our results suggest how interactions mediated by *glp-1* and *apx-1* contribute to the establishment of the dorsal-ventral axis in the early C. elegans embryo.**

## Introduction

The bodies of most animals have distinct anterior–posterior and dorsal–ventral asymmetries, and many also show distinct left–right asymmetries. Studies on early Drosophila embryos have provided insight into the molecular basis for axis formation in fruit flies. In Drosophila, an asymmetrical distribution of maternal products in the oocyte appears to prefigure both of the major axes of the body (reviewed by St Johnston and Nüsslein-Volhard, 1992). For example, the mRNA product of the *nanos* gene is localized at the posterior end of the Drosophila oocyte (Wang and Lehmann, 1991). The mislocalization of *nanos* mRNA to the anterior pole of the embryo results in the development of posterior pattern in place of anterior pattern (Wharton and Struhl, 1989; Gavis and Lehmann, 1992). The product of the *bicoid* gene is localized to the anterior pole of the Drosophila oocyte, where it functions to establish anterior pattern (Berleth et al., 1988; Driever et al., 1990). Drosophila oocytes also develop an asymmetrical distribution of *gurken* mRNA that corresponds to (and may define) the dorsal axis of the body (Neuman-Silberberg and Schüpbach, 1993).

The nematode Caenorhabditis elegans has distinct anterior–posterior, dorsal–ventral, and left–right axes. However, only the anterior–posterior axis appears to be correlated with an asymmetrical distribution of maternal gene products. There are several anterior–posterior asymmetries within the fertilized C. elegans egg that are visible prior to the first division. For example, cytoplasmic structures called P granules are segregated exclusively to the posterior pole of the egg (Strome and Wood, 1982). Shortly after the first division, the protein product of the maternal gene *skn-1* begins to accumulate at much higher levels in the posterior blastomere than in the anterior blastomere (Bowerman et al., 1993). The unequal distribution of these and other factors probably accounts for the very different properties of the anterior and posterior blastomeres after the first cleavage (Laufer et al., 1980; Cowan and McIntosh, 1985; Edgar and McGhee, 1986; Priess and Thomson, 1987; Schierenberg, 1987).

Dorsal–ventral asymmetries in the C. elegans embryo are first visible at the 4-cell stage. The dorsal-most blastomere, called ABp, undergoes an essentially invariant pattern of development that is very different from its more ventral sister, ABa (Sulston et al., 1983). However, at the beginning of the 4-cell stage, the fates of ABp and ABa can be switched by simply interchanging their positions relative to the other 4-cell stage blastomeres, called EMS and P2 (Priess and Thomson, 1987). These studies demonstrate that the dorsal–ventral axis is not determined before the 4-cell stage in C. elegans embryogenesis. ABp and ABa descendants undergo abnormal patterns of development if EMS or P2 is removed or destroyed with a laser microbeam (Priess and Thomson, 1987; Schnabel, 1991; Bowerman et al., 1992b), suggesting that interactions between these blastomeres (or their descendants) contribute to the different fates of ABa and ABp.

One difference between ABp and ABa is that only ABa descendants are induced to produce pharyngeal cells. Pharyngeal induction requires maternal expression of the *glp-1* gene (Priess et al., 1987). The GLP-1 protein and the homologous proteins LIN-12 (Yochem and Greenwald, 1989; Austin and Kimble, 1989; Greenwald, 1985; Yochem et al., 1988) and Notch (Artavanis-Tsakonas et al., 1983; Wharton et al., 1985; Kidd et al., 1986), appear to function as receptors for intercellular signals (reviewed by Greenwald and Rubin, 1992). Cell–cell interactions between ABa and EMS descendants are required for pharyngeal induction (Priess and Thomson, 1987). At the 12-cell stage, the MS blastomere, a daughter of EMS, contacts two ABa descendants (see Figure 1), which it induces to produce pharyngeal cells (H. Hutter and R. Schnabel, personal communication). Two additional ABa descendants are present at the 12-cell stage, but do not contact MS and are not induced to produce pharyngeal cells (see Figure 1). However, if the positions of the early blastomeres are altered such that these latter two ABa descendants contact MS, they appear to produce pharyngeal tissue (Wood, 1991; Wood and Kershaw, 1991). Thus, at the 12-cell stage, all of the ABa descendants appear to have the potential to interact with the MS blastomere. In contrast, none of the ABp descendants produce pharyngeal cells, although three ABp descendants contact MS at the 12-cell stage. The observations that ABa and ABp initially are equivalent in potential and that both ABa and ABp contact EMS and its descendants have raised the question of why

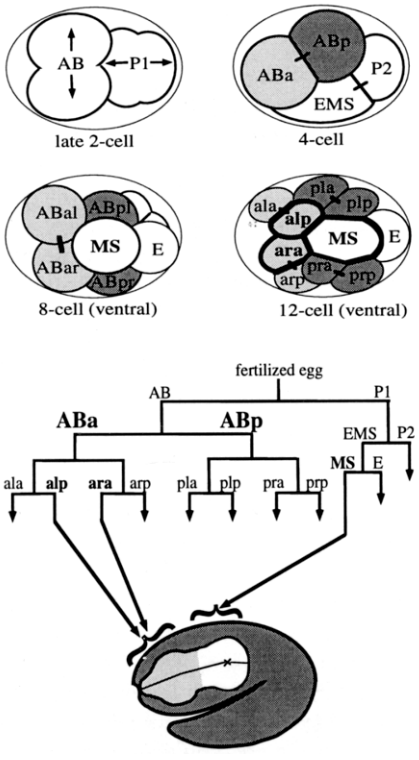


Figure 1. Cleavage Patterns and Fates of Early Blastomeres

Schematic drawings of the early C. elegans blastomeres are shown at the 2-cell, 4-cell, 8-cell, and 12-cell stages, with the anterior pole on the left. The 2-cell and 4-cell stages are viewed from their lateral surfaces, and the 8-cell and 12-cell stages are viewed from their ventral surfaces. Late in the 2-cell stage, the AB and P1 mitotic spindles (double-headed arrows) form. As the AB mitotic spindle grows in size, the newly formed AB daughters, ABa (light stippling) and ABp (dark stippling), are forced into different patterns of contact with the P1 daughters, EMS and P2. ABp is the dorsal-most blastomere, and EMS is the ventral-most blastomere. At the end of the 4-cell stage, ABa and ABp divide at right angles to both the anterior–posterior axis and the dorsal–ventral axis, and EMS divides parallel to the anterior–posterior axis. Thus, in the ventral view of the 8-cell stage, the daughters of ABa (called ABal and ABar) and the daughters of ABp (called ABpl and ABpr) flank one of the EMS daughters, called the MS blastomere. At the end of the 8-cell stage, the ABa and ABp daughters are the first to divide, resulting in a 12-cell stage embryo. The names of the ABa and ABp granddaughters are abbreviated in the 12-cell stage drawing and in the lineage diagram below; for example, the ABa daughter ABala is shown as a lightly stippled cell labeled ala. The 12-cell stage drawing is simplified to show only the contacts that the ABa and ABp granddaughters make with the MS and E blastomeres; the blastomeres that produce pharyngeal cells are shown in bold. An abbreviated version of the early embryonic cell lineage of C. elegans is shown to indicate the lineal origins of the 12-cell stage blastomeres. The fates of some of these blastomeres are represented in the highly schematic drawing of a embryo midway through development (compare with light micrograph of wild-type embryo in Figure 2a). The body of the embryo is shown in dark stippling to indicate that ABp descendants form most of the hypodermal covering of the body. The pharynx is the only internal organ represented in this drawing; the anterior half of the pharynx is shown in light stippling to indicate that the anterior pharyngeal cells originate primarily from ABa descendants, and the posterior half is left unstippled to indicate that posterior pharyngeal cells originate from the MS blastomere. The lineage diagram shows that ABalp and ABara (bold lettering) are the only ABa descendants that produce pharyngeal cells, and the drawing of the 12-cell stage embryo shows that these are the only ABa descendants that contact the MS blastomere.

only ABa is induced to produce pharyngeal tissue (Horvitz and Herskowitz, 1992).

In this paper we provide evidence that in wild-type development the ABa and ABp fates are established by two temporally and genetically distinct interactions. In embryos that fail to undergo the first of these interactions, the ABp fate is not specified, and both ABa and ABp descendants are induced to produce pharyngeal tissue. In embryos that fail to undergo the second interaction, neither ABa nor ABp descendants produce pharyngeal tissue. The second interaction, as previously shown, requires maternal *glp-1(+)* activity and involves descendants of the ABa and EMS blastomeres (Priess et al., 1987; Priess and Thomson, 1987). We show that the first interaction also requires maternal *glp-1(+)* activity and the activity of a second maternal gene, called *apx-1* (for anterior pharynx excess), that can encode a protein homologous to the Delta protein of Drosophila. In addition, we show that this first interaction requires contact between the ABp and P2 blastomeres. We propose a model for how the early cell–cell interactions mediated by the products of *glp-1* and *apx-1* contribute to the different fates of ABp and ABa and thus establish the dorsal–ventral axis of the early C. elegans embryo.

## Results

### Mutations in the *apx-1* Gene Result in a Hyperinduction of Pharyngeal Tissue

The pharynx of C. elegans is formed through two developmental pathways: ABa descendants form most of the anterior half of the pharynx through *glp-1*-mediated cell–cell interactions, and MS descendants form the posterior half of the pharynx independent of *glp-1(+)* activity (Figure 1; Sulston et al., 1983; Priess and Thomson, 1987; Priess et al., 1987). To determine why ABa and MS are the only blastomeres that produce pharyngeal cells, we have screened for mutants that overproduce pharyngeal cells and have described previously an analysis of genes that are required for the MS pathway (Mello et al., 1992). We have identified three recessive maternal-effect lethal mu-

Table 1. Pharyngeal Induction in *apx-1* and *glp-1** Mutant Embryos

| | Embryos with Pharyngeal Cells | | |
|---|---|---|---|
| Blastomere(s) Killed | Wild Type | *apx-1* | *glp-1** |
| EMS | 0 of 26 | 0 of 33 | 0 of 41 |
| ABa plus MSa/p | 0 of 27 | 28 of 31 | 16 of 16 |
| ABp plus MSa/p | 14 of 14 | 17 of 17 | 12 of 12 |
| ABa plus ABp plus MSa/p | ND | 0 of 11 | 0 of 5 |

Blastomeres were killed in wild-type, *apx-1(zu183)*, and *glp-1(e2141ts)* mutant embryos. *glp-1* embryos were maintained at nonpermissive temperature only until the 12-cell stage (*glp-1**). EMS is the parent of the MS blastomere (see Figure 1). MSa/p indicates that the MS blastomere was allowed to divide, and then both of its daughters (MSa and MSp) were killed. The resulting partial embryos were fixed and stained with the 3NB12 antibody to identify pharyngeal muscles. ND, not determined.

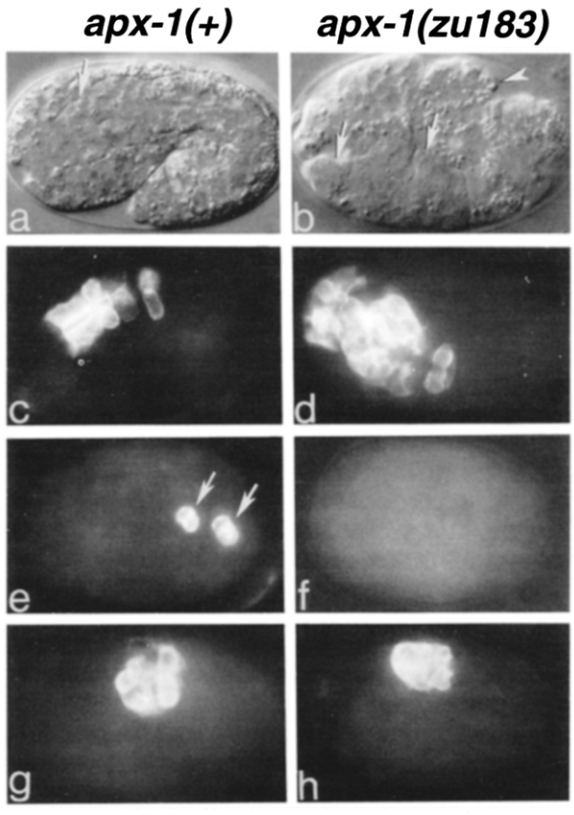


Figure 2. Differentiation in *apx-1(+)* and *apx-1(zu183)* Embryos

(a and b) Light micrographs of a wild-type embryo (a) and an *apx-1(zu183)* mutant embryo (b) showing the developing pharynx (arrows). Hypodermal cells surround and enclose the body of a wild-type embryo, but aggregate on one side of *apx-1* mutant embryos (arrowhead in [b]).

(c and d) Immunofluorescence micrographs of pharyngeal muscles. Most of the pharyngeal cells in the wild-type pharynx are visible in the single focal plane shown (c), single focal planes in *apx-1* mutant embryos show excess pharyngeal tissue (d), and additional pharyngeal cells are present in upper and lower focal planes (data not shown).

(e and f) Immunofluorescence micrographs of intestinal valve cells.

(g and h) Immunofluorescence micrographs of pharyngeal muscles. The embryo shown in (g) is from a mother homozygous for a *glp-1(e2141ts)* mutation and was maintained at nonpermissive temperature throughout embryogenesis. The embryo shown in (h) is from a mother homozygous for *glp-1(zu24);apx-1(zu183)*.

The pharyngeal muscles and intestinal valve cells shown in all the figures in this paper were stained with the monoclonal antibodies 3NB12 (Priess and Thomson, 1987) and ICB4 (Bowerman et al., 1992b), respectively. All images are at the same magnification; C. elegans eggs are about 50 μm in length.

tations in the gene *apx-1* that result in embryos with nearly twice the normal number of pharyngeal cells (Figure 2; see Experimental Procedures for genetic analysis of *apx-1* mutations). The following experiments demonstrate that the extra pharyngeal cells in *apx-1* mutant embryos result from an ABa-like, rather than an MS-like, developmental pathway.

Because MS does not require *glp-1(+)* activity to produce pharyngeal cells, the size of the pharynx in embryos carrying a mutation in the *glp-1* gene suggests how many pharyngeal cells are produced by MS-like blastomeres.

Table 2. Intestinal Valve Cell Development

| Wild-Type Embryos | *apx-1* Embryos | *glp-1* Embryos | *glp-1** Embryos | *glp-1* (Upshifted) Embryos |
|---|---|---|---|---|
| 12 of 12 | 0 of 23 | 2 of 17 | 0 of 24 | 17 of 17 |

*glp-1(e2141ts)* embryos were maintained at nonpermissive temperature throughout development. *glp-1** embryos were shifted from nonpermissive to permissive temperature at the 12-cell stage. *glp-1* (upshifted) embryos were shifted from permissive to nonpermissive temperature at the 12-cell stage. Embryos were stained with the ICB4 antibody to identify intestinal valve cells, and the EMS blastomere was killed in each experiment to prevent staining of the EMS-derived intestinal cells (Bowerman et al., 1992b).

We found that *glp-1;apx-1* double mutant embryos (Figure 2h) have much less pharyngeal tissue than *apx-1* single mutant embryos (Figure 2d) but amounts similar to *glp-1* single mutant embryos (Figure 2g). This result suggests that the MS blastomere in *apx-1* mutants produces the same number of pharyngeal cells as a wild-type MS and that the production of excess pharyngeal tissue in *apx-1* mutants requires *glp-1(+)* activity. In wild-type development, ABa descendants cannot produce pharyngeal cells if the MS blastomere (or MS precursors) are destroyed (Priess and Thomson, 1987; Bowerman et al., 1992a; Hutter and Schnabel, submitted). We found that *apx-1* mutant embryos did not produce pharyngeal cells if the parent of MS (the EMS blastomere) was destroyed with a laser microbeam (Table 1). Thus, *apx-1* mutant embryos appear to have a hyperinduction of pharyngeal tissue resulting from *glp-1(+)* activity and interactions involving the descendants of EMS.

### Mutations in the *glp-1* Gene Can Result in a Hyperinduction of Pharyngeal Tissue

Previously described mutations in the *glp-1* gene result in embryos with no induced pharyngeal tissue (Priess et al., 1987; Austin and Kimble, 1987). From the nearly reciprocal phenotypes of *glp-1* and *apx-1* mutant embryos, it is possible to imagine that the *glp-1* and *apx-1* genes have separate and opposite roles in permitting or preventing pharyngeal induction in wild-type embryogenesis. However, we noticed that *glp-1* and *apx-1* mutant embryos also have some phenotypic similarities; in both types of embryo, the hypodermal cells fail to enclose the body (Figure 2b; data not shown), and both types of mutant embryo fail to produce the intestinal valve cells, a cell type normally made by the ABp blastomere (Table 2). These observations suggested the possibility that *glp-1(+)* and *apx-1(+)* might act together in some cell fate decisions.

Previous analyses of temperature-sensitive *glp-1* mutations had demonstrated that embryonic viability required *glp-1(+)* activity between the 4-cell and 28-cell stages (Austin and Kimble, 1987; Priess et al., 1987). We performed a series of temperature-shift experiments on *glp-1(e2141ts)* mutant embryos to ask when *glp-1(+)* activity is required for pharyngeal induction, body morphogenesis, and intestinal valve cell specification. We reasoned that if *glp-1(+)*

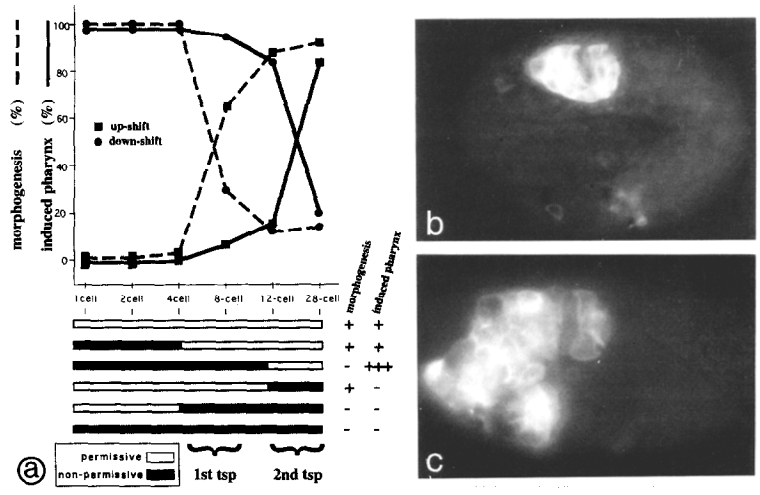


Figure 3. *glp-1(e2141ts)* Mutants Have Two Temperature-Sensitive Periods

(a) Temperature-shift experiments were performed on embryos (n > 40 per timepoint) from mothers homozygous for *glp-1(e2141ts)*. Selected temperature-shift protocols and results are indicated by the horizontal bars in the lower panel.

(b and c) Immunofluorescence micrographs of pharyngeal muscles in two temperature-shifted *glp-1(e2141ts)* embryos. The embryo in (b) was kept at permissive temperature until the 12-cell stage and then shifted up to the nonpermissive temperature. This *glp-1* embryo has substantially normal body morphogenesis, but does not appear to have any induced pharyngeal tissue (scored as minus); the small, partial pharynx in this embryo appears identical to the MS-derived partial pharynx described previously in *glp-1* mutant embryos (Priess et al., 1987). The embryo in (c) was kept at nonpermissive temperature until the 12-cell stage and then shifted down to the permissive temperature (these temperature-shifted embryos are referred to as *glp-1** embryos in the text). This embryo has a hyperinduction of pharynx (scored as triple plus) and appears very similar to *apx-1* mutant embryos (compare with Figure 2d).

functions with *apx-1(+)* at a specific stage in early embryogenesis, removing *glp-1(+)* activity only during that stage should result in an *apx-1*-like phenotype. The temperature-shift data are shown in Figure 3a and can be summarized by describing the results from the following three experiments. First, *glp-1* mutant embryos were maintained at the nonpermissive temperature throughout embryonic development. These embryos have no pharyngeal induction and produce only a small, MS-derived partial pharynx, as described previously (Priess et al., 1987). In addition, these embryos have severely defective body morphogenesis (data not shown). Second, *glp-1* mutant embryos were placed at the permissive temperature until the 12-cell stage and then shifted up to the nonpermissive temperature. These embryos also lack induced pharyngeal tissue, but have fairly normal body morphogenesis (Figure 3b). Some *glp-1* mutant embryos with similar defects also were observed in the temperature-shift experiments of Austin and Kimble (1987) using the allele *glp-1 (q231ts)*. Third, *glp-1* mutant embryos were kept at the nonpermissive temperature until the 12-cell stage and then shifted down to the permissive temperature for the remainder of development. We will refer to *glp-1* mutant embryos subjected to this third temperature-shift protocol several times in this paper and so, for simplicity, define these as *glp-1** embryos. We found that *glp-1** mutant embryos not only produce more pharyngeal tissue than *glp-1* mutants maintained at nonpermissive temperature throughout embryogenesis, but indeed produce more pharyngeal tissue than wild-type embryos (compare the *glp-1** embryo in Figure 3c with the wild-type embryo in Figure 2c). These *glp-1** mutant embryos closely resemble *apx-1* mutant embryos (see Figure 2d) in the amount of pharyngeal tissue they have and in their defects in body morphogenesis (data not shown). In addition, *glp-1** mutant embryos lack intestinal valve cells (Table 2), as do *apx-1* mutant embryos (see Figure 2f; Table 2). We found that *glp-1** mutant embryos, like *apx-1* mutant embryos, do not produce pharyngeal cells after the parent of the MS blastomere is destroyed with a laser microbeam (see Table 1). These observations suggest that *glp-1** mutant embryos, like *apx-1* mutant embryos, have a hyperinduction of pharyngeal tissue that requires signaling by the MS blastomere and *glp-1(+)* activity between the 12-cell and 28-cell stages.

### *apx-1* and *glp-1* Mutants Have ABp into ABa Transformations in Cell Fate

To determine the origins of the induced pharyngeal cells in the *apx-1* and *glp-1** mutant embryos, we used a laser microbeam to destroy various combinations of early blastomeres and asked whether the resulting embryos contained pharyngeal cells. In a wild-type embryo, if both daughters of the inducing cell (the MS blastomere) are killed after pharyngeal induction is completed, only ABa descendants produce pharyngeal cells (see Table 1). In contrast, we found that ABp descendants, in addition to ABa descendants, could produce pharyngeal cells after similar experiments on *apx-1* and *glp-1** mutant embryos (see Table 1).

The observation that ABp descendants in *apx-1* and *glp-1** mutant embryos can be induced to produce pharyngeal cells indicates that these descendants have at least one ABa-like characteristic. We therefore asked whether ABp and ABa descendants might have additional similarities by following the development of individual ABp and ABa descendants in *apx-1*, *glp-1**, and *glp-1* mutant embryos. These results are summarized in Figure 4a, and examples of this analysis are described below. Although the blastomere names and patterns of differentiation are complicated, the major conclusion is very simple: in *apx-1*, *glp-1**, and *glp-1* mutant embryos, ABp descendants adopt the fates of wild-type ABa descendants. The transformations in cell fate that we describe involve lineally equivalent cells. Cells that have different origins can be considered to be lineally equivalent if they share an analogous pattern

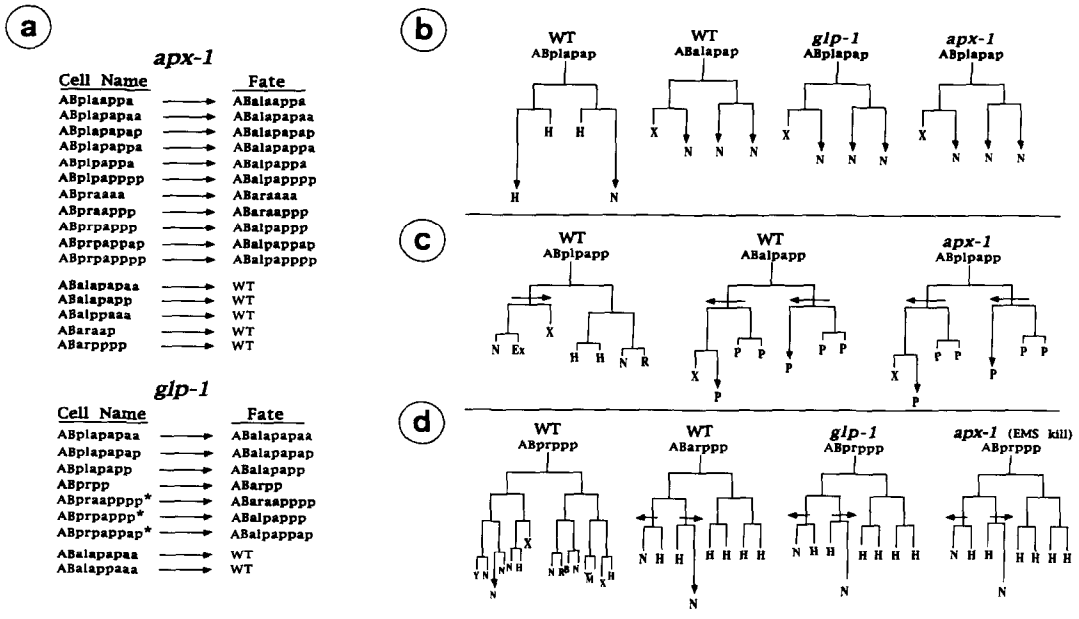


Figure 4. Lineage Transformations in *apx-1* and *glp-1* Mutant Embryos

(a) The names of the ABp and ABa descendants examined in *apx-1(zu183)* or *glp-1(e2141ts)* mutant embryos are listed, and the fates of these cells are summarized. If a cell in a mutant embryo had the same pattern of development it has in wild-type embryogenesis, its fate is shown as WT. If a cell instead appeared to have an ABp into ABa transformation in fate, the fate of the corresponing ABa descendant is listed. For example, ABplaappa in *apx-1* embryos does not develop like a wild-type ABplaappa, but instead appears similar to a wild-type ABalaappa. The fates of three ABp descendants were determined in the *glp-1** mutant embryo; these are ABpraapppp*, ABprpappp*, and ABprpappap*.

(b–d) Examples of three representative lineages in wild-type, *glp-1*, and *apx-1* embryos. Horizontal lines indicate cell divisions, with the anterior-most daughter cell on the left and the posterior-most daughter on the right. The relative times at which cell divisions occur are indicated by the positions of the horizontal lines across the vertical axis. Horizontal arrows above a cell division indicate that the daughter cells had very different sizes; the arrow points toward the smaller daughter. Lineage data for wild-type embryos are taken from Sulston et al. (1983). H, hypodermal cell; N, neuron; X, cell death; Ex, excretory cell; R, rectal epithelial cell; P, pharyngeal cell; Y and B, postembryonic blast cells; M, muscle.

of birth; in C. elegans nomenclature, this pattern of birth (or lineage) is indicated by the name of a cell. For example, ABp and ABa both undergo left–right divisions, and their left-hand daughters are called ABpl and ABal, respectively. Because ABpl and ABal result from a similar lineage pattern, these blastomeres can be described as lineally equivalent descendants of ABp and ABa.

In wild-type development, an ABa descendant called ABalapap produces cells that differentiate as neurons or that undergo cell death (Figure 4b; Sulston et al., 1983). We found that ABalapap descendants in *apx-1* and *glp-1* mutant embryos also have a wild-type pattern of development (Figure 4a). In wild-type development, ABalapap and the lineally equivalent descendant of ABp, called ABplapap, have very different patterns of division and differentiation (Figure 4b). In *apx-1* and *glp-1* mutant embryos, we found that ABplapap did not develop like a wild-type ABplapap, but instead had a pattern of development very similar to ABalapap (Figure 4b). The blastomeres ABplpapp and ABalpapp provide a second example of lineally equivalent ABp and ABa descendants. In *apx-1* mutant embryos, we found that ABplpapp descendants undergo patterns of division and differentiation that are very different from those of a wild-type ABplpapp blastomere but very similar to those of a wild-type ABalpapp blastomere (Figure 4c).

An exception to the simple pattern of ABp into ABa transformations in fate in *apx-1* and *glp-1** embryos is the development of the ABprp blastomere. ABprp is lineally equivalent to an ABa descendant called ABarp; however, we found that ABprp descendants appeared to develop like the descendants of ABalp instead of ABarp (Figure 4a; see Discussion). Because ABprp contacts the MS blastomere, but ABarp does not (see Figure 1), it was possible that inductive interactions with MS prevent ABprp from developing like ABarp. This hypothesis predicts that ABprp would adopt the fate of ABarp in *apx-1* and *glp-1** mutants if pharyngeal induction was prevented. To test this idea, we prevented pharyngeal induction in *apx-1* mutants by killing the parent of MS (the EMS blastomere). Because pharyngeal induction requires *glp-1(+)* activity, we prevented pharyngeal induction in the *glp-1* mutants by simply keeping these embryos at nonpermissive temperature throughout development. Under these experimental conditions, we found that descendants of ABprp in both *apx-1* and *glp-1* mutant embryos adopt fates similar to those of ABarp descendants (Figure 4d). These results suggest that the ABprp blastomere in *apx-1* and *glp-1** mutant embryos has an inherent potential identical to ABarp, but that ABprp adopts an alternative fate because of interactions with the MS blastomere. In summary, all of the ABp descendants that we examined in *apx-1*, *glp-1**, and *glp-1* mutant embryos have developmental properties that are different from wild-type ABp descendants, but similar or identical to those of wild-type ABa descendants. There-

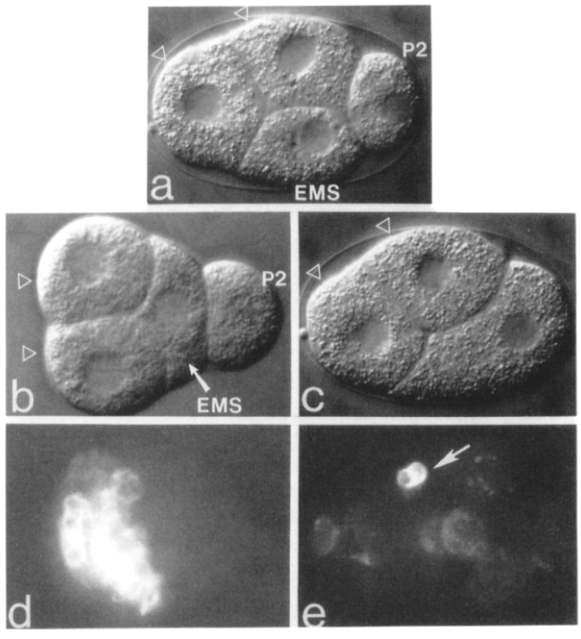


Figure 5. Differentiation in Wild-Type Embryos with Altered Contacts between Early Blastomeres

(a) Light micrograph of a normal 4-cell stage embryo (compare with diagram in Figure 1). The sister blastomeres ABa and ABp are indicated by open arrowheads, and the sister blastomeres P2 and EMS are labeled. Note that ABp, but not ABa, contacts P2.

(b) Light micrograph of a 4-cell stage embryo after removing the outermost layer of the eggshell. In this embryo, neither ABp nor ABa contacts the P2 blastomere, but both contact EMS.

(c) Light micrograph of embryo in which the parent of the P2 and EMS blastomeres is prevented from dividing. The undivided blastomere contacts both ABa and ABp.

(d) Immunofluorescence micrograph of pharyngeal cells in the embryo shown in (b) at the completion of embryonic development. The embryo produced more pharyngeal tissue than a normal embryo (compare with Figure 2c), and pharyngeal tissue was visible on several different focal planes in this embryo (data not shown).

(e) Immunofluorescence micrograph of valve cells produced by ABa in the embryo shown in (c). At the end of the cell cycle shown in (c), the ABp blastomere and the undivided parent of P2 and EMS were destroyed with a laser microbeam, allowing only the ABa blastomere to develop.

fore, *apx-1(+)* and *glp-1(+)* activities appear to be required for ABp to adopt a fate different from ABa.

### Blastomere Contacts and Specification of the ABp Fate

Our analysis of temperature-shifted *glp-1* mutant embryos indicates that *glp-1(+)* activity is required between the 4-cell and 12-cell stages for ABp to adopt a fate different from its sister, the ABa blastomere. The *glp-1* gene product and homologous genes in C. elegans and Drosophila are known to function in several different cell–cell interactions (reviewed by Greenwald and Rubin, 1992). These observations suggest that there is likely to be a cell–cell interaction between ABp and some other embryonic blastomere. If this interaction were prevented, ABp would be predicted to have developmental defects similar to those seen in *glp-1** and *apx-1* mutant embryos; for example, ABp descendants should be induced to produce pharyngeal cells.

To test which blastomeres might interact with ABp, we used a laser microbeam to irradiate each of the blastomeres in embryos between the 4-cell and 12-cell stages. ABp did not produce pharyngeal cells in any of these embryos (data not shown). Therefore, this experimental procedure did not indicate which, if any, of the early blastomeres interact with ABp. However, the laser-irradiated blastomeres remain in contact with ABp in these experimental embryos and might still be capable of interacting. For this reason, we examined the development of ABp in embryos in which the pattern of blastomere contacts had been altered.

In normal 4-cell stage embryos, ABp and ABa have asymmetrical contacts with the neighboring blastomeres EMS and P2: both ABp and ABa contact EMS, but only ABp contacts P2 (Figure 5a; see also Figure 1). Previous studies had shown that the outermost layer of the eggshell surrounding the C. elegans embryo plays a role in establishing these asymmetrical contacts; when this eggshell layer is removed enzymatically, both ABp and ABa contact EMS, as in normal embryos, but neither ABp nor ABa contacts the P2 blastomere (Hyman and White, 1987). These embryos have a symmetrical, T-shaped configuration, in contrast with normal embryos, which are rhombohedral in shape. We removed the outer layer of the eggshell from 2-cell stage wild-type embryos and observed the development of those embryos that were symmetrically T shaped at the beginning of the 4-cell stage (Figure 5b). Many of the initially T-shaped embryos establish the normal pattern of blastomere contacts during the 4-cell stage and hatch into worms (Wood and Kershaw, 1991; Schierenberg and Junkersdorf, 1992). However, we found that approximately 30% of the embryos remain T shaped throughout the 4-cell stage ($n > 200$) and that these embryos do not develop into viable animals. This latter group of embryos was allowed to develop to terminal stages, examined in the light microscope, and then fixed and stained with pharyngeal-specific antibodies. We found that these embryos do not undergo body morphogenesis and often appear to overproduce pharyngeal cells (Figure 5d). We repeated this analysis on T-shaped *glp-1(e2141ts)* mutant embryos and found that at the nonpermissive temperature, these *glp-1* embryos do not overproduce pharyngeal cells ($n > 50$). Therefore, the extra pharyngeal cells observed in T-shaped wild-type embryos appear to result from *glp-1*-mediated pharyngeal induction.

To determine the origins of the extra pharyngeal cells, we observed the development of ABp and ABa descendants in five T-shaped wild-type embryos. In three of these embryos, we were able to follow ABp and ABa descendants simultaneously. In each case, ABp and ABa both produced descendants that differentiated into pharyngeal cells (data not shown). In the other two embryos examined, it was not possible to follow both sister blastomeres simultaneously, but the blastomere observed produced pharyngeal cells in each case. The blastomeres selected for lineage analysis in the five T-shaped embryos were lineally

equivalent to the ABalp blastomere, which produces pharyngeal cells in wild-type development (see Figure 1). In each of these embryos, the blastomeres examined had a pattern of division and differentiation similar to that of ABalp descendants (data not shown). Thus, in the T-shaped embryos, both ABa and ABp appear to have similar patterns of development and to resemble wild-type ABa blastomeres. These results suggest that ABp interacts with the P2 blastomere and that these interactions allow ABp to become different from ABa.

If interactions with P2 are required to specify the ABp fate, then the ABa blastomere might also produce ABp-like descendants if it contacted the P2 blastomere during the 4-cell stage. One marker of ABp-specific differentiation is the presence of intestinal valve cells, which are made only by ABp in normal development (Sulston et al., 1983). We have shown that *glp-1(+)* activity between the 4-cell and 12-cell stages is required for ABp to produce the valve cells, and previous studies demonstrated that interactions were required between ABp and P2 (or their descendants) for ABp to produce valve cells (Bowerman et al., 1992b). To test whether ABa also could produce valve cells if it contacted P2, we prevented the parent of the P2 blastomere from dividing by irradiating its centrosome with a laser microbeam. ABa and ABp are born at the normal time and in the correct positions in these embryos, but both ABa and ABp contact the undivided parent of P2 throughout the next cell cycle, corresponding to the 4-cell stage (Figure 5c). These embryos were allowed to develop to terminal stages and were then fixed and stained with an antibody that recognizes the intestinal valve cells. We found that these embryos produce more valve cells than normally are made by ABp (data not shown). To confirm that ABa was the source of the extra valve cells, we prevented the P2 parent from dividing and then killed the ABp blastomere. We found that several of the resulting embryos contained valve cells (n = 4 of 11; Figure 5e), indicating that the ABa blastomere can produce valve cells in these experiments. This result suggests that the same *glp-1*-mediated interactions that occur between ABp and P2 in normal embryogenesis can occur between ABa and the undivided P2 parent.

### The *apx-1* Gene Can Encode a Delta-like Protein

We cloned the *apx-1* gene to begin a molecular analysis of its function in the cell–cell interactions between ABp and P2. We found that the *apx-1(zu183)* mutation, which originated spontaneously in a mutator strain, comapped with a new insertion of the transposon Tc1 (see Experimental Procedures). DNA flanking the Tc1 insertion site was isolated and used as a probe to identify potential *apx-1* genomic and cDNA clones (Figure 6). The cosmid C40A5 contains genomic sequences that are polymorphic in two different *apx-1* mutant strains, and the C40A5 cosmid clone fully rescues the *apx-1(zu183)* mutation (see Experimental Procedures). The rescuing sequences and the two *apx-1* allele polymorphisms shown in Figure 6 define an ~15 kb genomic interval. A 2.1 kb cDNA clone hybridizes throughout this 15 kb region, with the inferred exon/intron structure shown in Figure 6A. Sequence analysis of this cDNA and of *apx-1(zu183)* genomic DNA indicates that the *zu183* mutation is a Tc1 insertion into the 3′ untranslated region of the predicted transcript (Figure 6B). Based on these data, we conclude that the 2.1 kb cDNA represents a product of the *apx-1* gene.

The *apx-1* cDNA clone can encode a transmembrane protein similar to the Delta and Serrate proteins of Drosophila and the LAG-2 protein of C. elegans (Figure 6; Vassin et al., 1987; Kopczynski et al., 1988; Fleming et al., 1990; Thomas et al., 1991; Tax et al., 1994). The sequence homology within this gene family lies in the presumptive extracellular domain in a region that contains epidermal growth factor–like motifs (Figures 6C and D).

## Discussion

At the beginning of the 4-cell stage, the ABp blastomere is equivalent to its sister, the ABa blastomere (Priess et al., 1987). However, ABp descendants undergo patterns of cell division and differentiation that are very different from those of lineally equivalent ABa descendants (Sulston et al., 1983). In this paper we have described three types of embryos in which ABp descendants adopt a pattern of cell division and differentiation that is very similar or identical to that of ABa descendants: *apx-1* mutant embryos, *glp-1* mutant embryos exposed to nonpermissive temperature only until the 12-cell stage (*glp-1** embryos), and wild-type embryos in which the P2 blastomere does not contact ABp. These results suggest that in wild-type embryogenesis, ABp requires maternally expressed *apx-1(+)* and *glp-1(+)* activities and contact with the P2 blastomere to become different from ABa.

### Maternally Expressed *glp-1* Functions in Two Distinct Interactions during the Early Cleavage Stages

The *glp-1* gene encodes a homolog of the C. elegans gene *lin-12* and the Drosophila gene *Notch* (Yochem and Greenwald, 1989; Yochem et al., 1988). Each of these genes has been shown to function in several different cell–cell interactions in the development of nematodes and fruit flies (reviewed by Greenwald and Rubin, 1992). For example, *glp-1* has been shown to function postembryonically in C. elegans in interactions that are required for the proper development of the germline (Austin and Kimble, 1987). Experimental analysis of the germline interaction (Kimble, 1981) and analysis of animals mosaic for *glp-1(+)* activity (Austin and Kimble, 1987) indicate that GLP-1 protein is likely to function in signal reception in a manner analogous to the function of the LIN-12 and Notch proteins.

Recent immunolocalization studies have shown that GLP-1 protein is on the surfaces of both ABa and ABp and on the surfaces of all of their descendants through the 28-cell stage of embryogenesis (S. Crittenden and J. Kimble, personal communication). Previous studies had shown that maternally expressed *glp-1(+)* activity is required for ABa development (Priess et al., 1987), but *glp-1(+)* was not known to be necessary for proper ABp

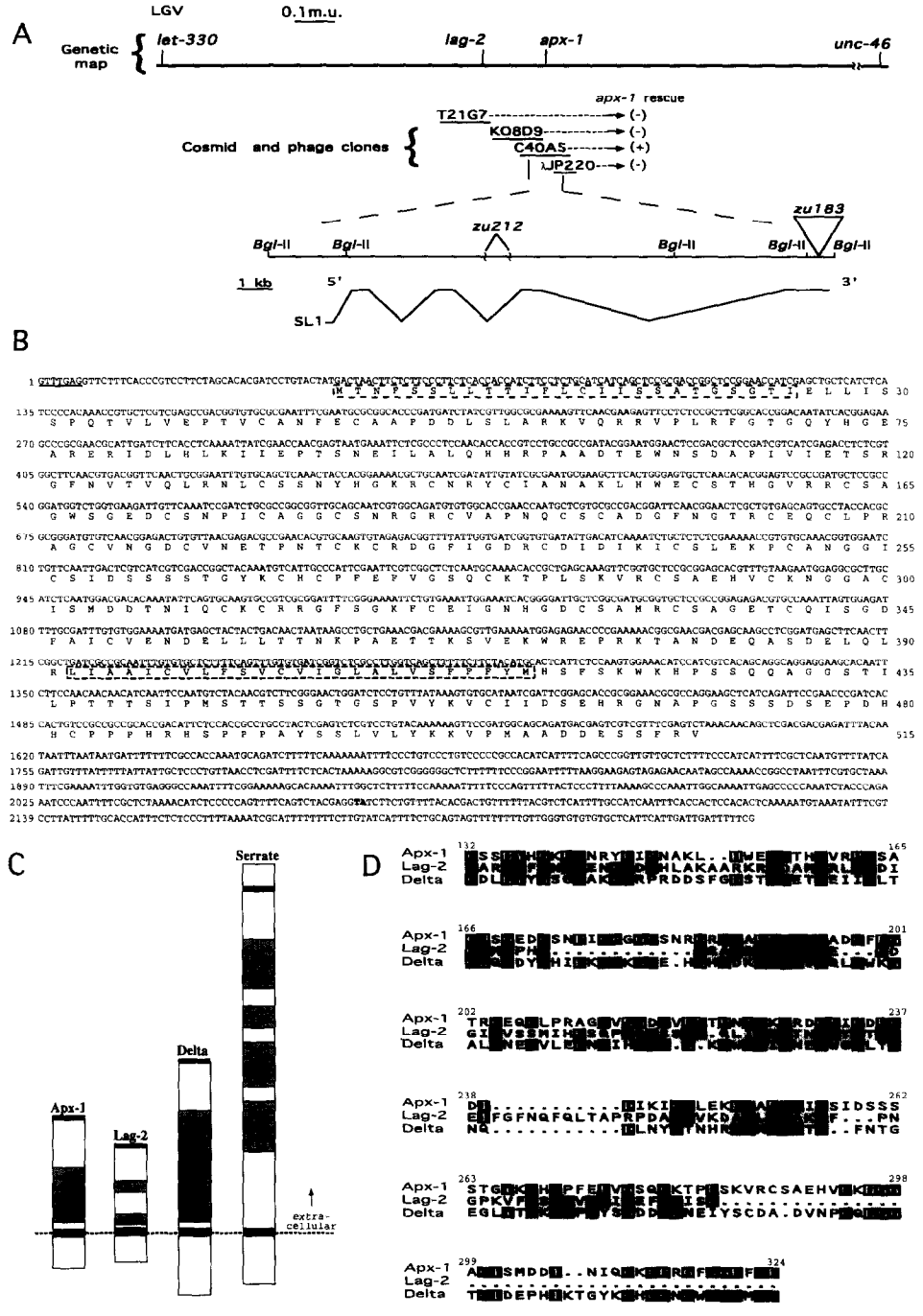


Figure 6. Molecular Cloning of *apx-1*

(A) Genetic and physical maps of the *apx-1* region on linkage group V (LGV). The positions of the *zu183* Tc1 insertion and of an ~900 bp deletion in the *zu212* allele are indicated. A 2.1 kb *apx-1* cDNA clone is shown beneath the genomic restriction map. The trans-spliced leader SL1 (Krause and Hirsh, 1987) is present on the 5′ end of the *apx-1* message (see Experimental Procedures).

(B) The nucleotide and predicted amino acid sequences from the *apx-1* cDNA. SL1 sequences are underlined at the 5′ end, and the last nucleotide in the cDNA sequence is followed by a poly(A) tail. The TA dinucleotide (position 2076) is the site of Tc1 insertion in *apx-1(zu183)*. Hydrophobic amino acid residues corresponding to a possible signal peptide and a transmembrane domain are boxed.

(C) Schematic diagrams of APX-1 and related proteins from Drosophila and C. elegans. The signal peptide and transmembrane domains are shown as closed boxes. Stippled boxes indicate the position of the epidermal growth factor motifs found in each family member.

(D) Sequence alignment of the epidermal growth factor–like regions in *apx-1* (residues 132–324) with the predicted products of the C. elegans gene *lag-2* (residues 124–269) and the Drosophila gene *Delta* (residues 184–372).

development. Our analysis of temperature-sensitive *glp-1* mutants indicates that maternally expressed *glp-1* is required for the proper specification of both blastomeres: *glp-1(+)* activity is required between the 4-cell and 12-cell stages for the normal pattern of ABp development and again between the 12-cell and 28-cell stages for proper development of ABa descendants. These observations suggest that maternally expressed GLP-1 protein functions in ABp and ABa descendants as a receptor for two different interactions during the early cleavage stages.

### The Specification of ABa

*glp-1(+)* activity between the 12-cell and 28-cell stages is required for interactions that result in a subset of ABa descendants producing pharyngeal cells (Priess and Thomson, 1987; Priess et al., 1987). The MS blastomere appears to function as the signaling cell in these interactions: pharyngeal induction does not occur if the MS blastomere is killed soon after its birth at the 8-cell stage (Hutter and Schnabel, submitted) or if any of the MS precursors are removed from the embryo or destroyed with a laser microbeam (Priess and Thomson, 1987; Bowerman et al., 1992a).

Two lines of evidence suggest that zygotic gene activity is required for the MS blastomere to function as a signaling cell. First, the gene *skn-1*, which encodes a probable transcriptional regulator, is required for the interactions between MS and ABa descendants (Bowerman et al., 1992a). At the 8-cell stage, SKN-1 protein is present in the MS nucleus (Bowerman et al., 1993), where it may function to control transcriptional events required for signaling. Second, we have found in the present study that it is very easy to prevent the interactions between MS and ABa descendants by small amounts of laser-induced damage to the nucleus of the parent of MS. These results suggest the possibility that zygotic gene activity in MS results in the expression or activation of a ligand for *glp-1*.

### The Specification of ABp

We have shown that *glp-1(+)* activity between the 4-cell and 12-cell stages is required for interactions that result in ABp adopting a fate different from ABa. The present study and results from previous work together suggest that the P2 blastomere functions as the signaling cell in these interactions. We have shown here that if the P2 blastomere never contacts the ABp blastomere, ABp adopts an ABa-like fate, resulting in embryos with a hyperinduction of pharyngeal tissue and no body morphogenesis. Previous studies have shown that if the P2 blastomere is allowed to contact ABp, but then removed later in the 4-cell stage, the resulting partial embryos often have normal amounts of pharyngeal tissue and undergo some body morphogenesis (Priess and Thomson, 1987). We interpret this latter result to mean that by the end of the 4-cell stage, ABp has become at least partially committed to the wild-type pattern of ABp-specific differentiation. Our observation that *glp-1(+)* activity is required beyond the 4-cell stage could indicate that interactions continue between ABp and P2 descendants until the 12-cell stage. Alternatively, P2 signaling could be completed at the 4-cell stage, but signal transduction could require *glp-1(+)* activity in ABp descendants until the 12-cell stage.

Our results suggest that the P2 signal is encoded by gene products that are expressed maternally rather than zygotically. We were unable to prevent interactions between the P2 and ABp blastomeres by even severe laser-induced damage to the P2 nucleus, suggesting that the gene products required for P2 signaling already may be present on the membrane (or in the cytoplasm) of the P2 blastomere. Mutations in the maternally expressed gene *apx-1* cause defects in the development of ABp that appear identical to the ABp defects observed in *glp-1* mutant embryos. The finding that *apx-1* can encode a protein with homology to Delta, a probable ligand for Notch in Drosophila (reviewed by Greenwald and Rubin, 1992), suggests that the product of *apx-1* may have an analogous role as a ligand for *glp-1*.

### Control of *glp-1(+)* Activity

We have shown that ABp, but not ABa, appears to undergo a *glp-1*-mediated interaction between the 4- and 12-cell stages. If *glp-1* does not function in ABa before the 12-cell stage, what keeps it from functioning? One possibility is that activation of the *glp-1* receptor requires signaling molecules that, until the 12-cell stage, are present or active in the P2 blastomere but not in its sister, the EMS blastomere (Figure 7). We prevented the division that normally generates P2 and EMS, allowing the undivided parental blastomere to contact ABa, and found that ABa was able to produce intestinal valve cells. Intestinal valve cells normally are made only by ABp, and we have shown that *glp-1(+)* activity between the 4-cell and 12-cell stages is required for valve cell specification. Thus, when the undivided parent of P2 and EMS contacts the ABa blastomere, ABa appears to undergo a *glp-1*-mediated interaction between the 4-cell and 12-cell stages (as ABp does in normal development). These results suggest that the division of P2 from EMS may result in P2 inheriting factors required for signaling that are not present in EMS.

We propose that the activity of the maternally expressed GLP-1 protein is regulated in the ABa and ABp blastomeres by differential exposure to ligand. Expression of the APX-1 protein on the surface of P2 (but not EMS) may result in *glp-1(+)* activity only in the adjacent ABp blastomere. Zygotic expression of a second *glp-1* ligand in the MS blastomere might later result in *glp-1(+)* activity in ABa descendants (Figure 7). The different responses of ABp

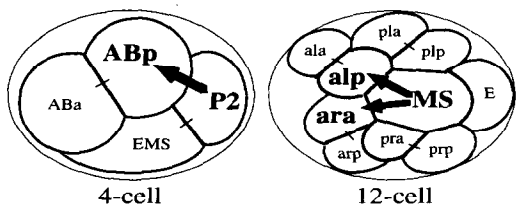


Figure 7. Cell–Cell Interactions at the 4-Cell and 12-Cell Stages

and ABa descendants to *glp-1(+)* activity may result from different downstream effector molecules expressed at the 4-cell and 12-cell stages.

Zygotically expressed genes that might encode the MS signal have not yet been identified. The product of the *lag-2* gene in C. elegans has been shown recently to encode a second Delta-like protein (Tax et al., 1994), and previous studies suggested that the product of *lag-2* was likely to function with *glp-1* in late embryogenesis (Lambie and Kimble, 1991). We have examined the phenotypes of embryos that were homozygous for chromosomal deficiencies removing either (or both) of the *apx-1* and *lag-2* genes. These deficiency embryos should lack all zygotic expression of the *apx-1* and *lag-2* genes, yet such embryos appear to have normal pharyngeal induction (our unpublished data). These results suggest the possibility that a third gene encoding a Delta-like protein exists in C. elegans. Alternatively, maternal products of *apx-1* or *lag-2* genes might function as the MS ligand, but the activity of these products may be controlled by zygotically expressed genes.

### The Left-Right Axis

Blastomere manipulation experiments by Wood (1991) have provided evidence that left–right asymmetries in the positions of the early blastomeres may determine their left–right asymmetries in fate. Our analysis of the development of the ABprp blastomere in *apx-1* and *glp-1** mutant embryos supports this view. We found that ABprp develops like the lineally equivalent ABa descendant in both mutant strains if the MS blastomere is killed. However, if the MS blastomere is not killed, ABprp does not develop like the lineally equivalent ABa descendant (called ABarp). Instead, ABprp, which is on the right-hand side of the embryo, develops in *apx-1* and *glp-1** mutants like a wild-type ABalp blastomere, which is on the left-hand side. This observation raises the possibility that in wild-type embryogenesis, ABalp differs from ABarp only because ABalp interacts with the MS blastomere and ABarp does not. In a recent study, H. Hutter and R. Schnabel (personal communication) followed the development of right and left pairs of ABa descendants in *glp-1* mutant embryos and in wild-type embryos after killing the MS blastomere with a laser microbeam; in these embryos, left- and right-handed pairs of blastomeres had identical patterns of development, supporting the hypothesis that *glp-1*-mediated interactions between MS and ABa descendants contribute to left–right asymmetries in cell fate. Thus, in C. elegans left–right asymmetries in the positions of otherwise identical blastomeres may place them in different locations with respect to signaling cells. These asymmetrical positions lead to asymmetrical patterns of cell–cell interactions and hence to left–right asymmetries in blastomere fate.

### Establishment of the Dorsal-Ventral Axis in C. elegans Embryos

The dorsal–ventral axis, like the left–right axis, appears to be established through cell–cell interactions during C. elegans embryogenesis; the dorsal–ventral axis can be inverted by changing the relative positions of the ABp and ABa blastomeres (Priess and Thomson, 1987). Therefore, the cell–cell interactions that make ABp different from ABa play a major role in determining the polarity of the dorsal–ventral axis of the embryo. Removing the outer layer of the eggshell allows ABp and ABa to occupy symmetrical positions with respect to other blastomeres, resulting in embryos that do not have any visible dorsal–ventral polarity. We have shown that dorsal fates (ABp fates) do not appear to be specified in these embryos. Instead, both ABp and ABa appear to adopt ventral fates (ABa fates), similar to the transformations in cell fate we observed for ABp descendants in *apx-1* and *glp-1** mutant embryos. Thus, in wild-type development, the eggshell plays a significant role in defining the dorsal–ventral axis by forcing ABp and ABa to adopt asymmetrical positions.

Our results suggest a general model for how preexisting asymmetries on the anterior–posterior axis may lead to the establishment of a second axis, the dorsal–ventral axis, during early C. elegans embryogenesis. We propose that the anterior and posterior blastomeres in the early embryo express different molecular components of a signaling pathway. The maternal product of the *glp-1* gene is likely to act as a receptor that is localized anteriorly to ABa and ABp. We propose that a ligand for *glp-1*, perhaps encoded by the *apx-1* gene, is localized to the posterior-most blastomere, P2. The dorsal–ventral asymmetry imposed by the eggshell causes ABp, but not ABa, to contact P2. The resulting *glp-1*- or *apx-1*-mediated interactions between ABp and P2 specify the ABp fate, ensuring proper dorsal–ventral patterning in subsequent development.

## Experimental Procedures

### Strains and Alleles

The strain N2 was used as the standard wild-type strain. The genetic markers, deficiencies, duplications, and balancer chromosomes used are listed by linkage group as follows: LGI, *dpy-5(e61)*; LGII, *bli-2(e768)*; LGIII, *unc-4(e120), glp-1(zu24), glp-1(e2141ts), glp-1(e2142ts), glp-1(q231ts), eT1(III;V)*; LGIV, *unc-5(e53)*; LGV, *apx-1(zu183), apx-1(zu212), apx-1(zu215), bgm-1(or3), dpy-11(e224), lag-2(n1255n1323), lag-2(s1486), lag-2(q387), lag-2(q431), let-330(s573), unc-46(e177), sDf26, sDf27, sDf31, sDf34, sDf40, sDf41, sDf48, sDf49, sDf51, yDp1*; LGX, *lin-2(e1309), lon-2(e678)*. The *bgm-1(or3)* mutation was isolated by B. Bowerman and fails to complement *apx-1*. *lag-2(s1486)* was formerly called *let-461* (Johnson and Baillie, 1991). *let-330* and the *sDf* chromosomal deficiencies were provided by B. Johnson and D. Baillie. Nematode strains were cultured by standard techniques (Brenner, 1974).

### Genetic Analysis

Maternal-effect lethal mutations were isolated using a so-called sib selection screen in a transposon-mobilized strain, RW7096[*mut-6(st702) unc-22(st192::Tc1)*] (Mori et al., 1988), made homozygous for *lin-2(e1309)*. Individual *mut-6;lin-2* hermaphrodites were allowed to grow over several generations to establish independent populations of animals. Eggs were purified from each population by treatment with bleach (Wood et al., 1988). After a 12 hr incubation, sets of 100 larvae were plated onto single 50 mm petri plates and allowed to grow through two generations. The plates were examined in the light microscope for the presence of adults filled with dead eggs, and these eggs were collected and examined with the compound microscope. Animals heterozygous for mutations of interest were recovered by plating and rescreening (sib selection). Heterozygous siblings with a mutation of interest were recovered after rescreening about 2000–6000 F3 larvae from the plate identified in the primary screen. Two *apx-1* alleles were obtained from a total of ~ $10^6$ haploid genomes screened. *apx-1(zu215)*

is an ethylmethane sulfonate-induced mutation isolated by R. Lin (personal communication) in a screen, following previously reported procedures (Brenner, 1974; Priess et al., 1987).

*apx-1(zu183)* was positioned with respect to *let-330*, *lag-2*, and *unc-46* on LGV by standard three-factor crosses. Data for these crosses are available from the C. elegans Genetic Stock Center. The deficiency *sDf70* complements *apx-1*, and the following deficiency strains fail to complement *apx-1*: *sDf26*, *sDf27*, *sDf31*, *sDf34*, *sDf40*, *sDf41*, *sDf48*, *sDf49*, *sDf51*. Two *lag-2* alleles, *lag-2(q387)* and *lag-2(q431)*, are chromosomal deficiencies (D. Gao, personal communication) that also fail to complement *apx-1*. Deficiency heterozygotes were generated as described in the following example. Homozygous *apx-1(zu183)* males were crossed to animals heterozygous for the *q387* deficiency balanced by the translocation *nT1*. The resulting *apx-1/q387* heterozygotes were viable and as adults produced wild-type numbers of eggs at 22°C (average, 182); these embryos arrested development with phenotypes indistinguishable from embryos produced by homozygous *apx-1* mutant mothers (data not shown).

Embryogenesis requires maternal expression of the *apx-1* gene, as shown by the following experiments. For maternal necessity, no viable self-progeny were produced by adult hermaphrodites homozygous for *apx-1* mutations (*zu183*, n = 3146; *zu212*, n = 607; *zu215*, n = 1064). Homozygous *apx-1(zu183)* hermaphrodites purged of self-sperm produced only inviable progeny (n > 1000) when mated to wild-type males. For maternal sufficiency, purged hermaphrodites heterozygous for *apx-1(zu183)* were mated to homozygous *apx-1(zu183)* males. These mated hermaphrodites produced embryos with wild-type levels of viability (98%; n = 485). The hatched larvae grew to fertile adults, indicating that paternal and zygotic expression of *apx-1* is not necessary for viability.

### Analysis of Embryos

Previously described protocols were followed for lineage analysis (Sulston et al., 1983), immunofluorescence microscopy (Albertson, 1984), laser ablation experiments (Bowerman et al., 1992a; Mello et al., 1992), temperature-shift experiments (Priess et al., 1987), and cell fate assignment (Mello et al., 1992). Embryos without outer eggshell layers were prepared for light microscopy as follows. The outer eggshell layers were removed from 1-cell and 2-cell stage embryos as described previously (Wolf et al., 1983). In brief, early embryos were treated with sodium hypochlorite, rinsed in M9 salts, and treated with about 100 μg/ml chitinase (Serratia marcescens, Sigma Incorporated) in isotonic media buffer. These embryos retain an inner eggshell layer, but are very fragile. The embryos were transferred in egg salts to microscope slides that were treated with poly-L-lysine (150,000 MW, Sigma). Coverslips (18 mm × 18 mm) were placed on each side of the embryos to serve as a support for a 22 mm × 50 mm coverslip that then was laid across the embryos for microscopy.

In wild-type development, there are several lineally equivalent descendants of ABa and ABp that have similar patterns of development. Therefore, we chose those ABa and ABp descendants that normally have very different patterns of development for lineage analysis. Most of the lineages were repeated at least twice in different embryos.

### Cloning of *apx-1*

Tc1 elements in the *apx-1(zu183)* strain were tested for linkage to the *apx-1* gene using standard three-factor mapping techniques. A single transposon comapped with *apx-1* in three recombinants within the *lag-2;apx-1* interval and 72 recombinants within the *apx-1;unc-46* interval. The transposon flanking sequences were recovered as described by Hill and Sternberg (1992). cDNA clones were isolated from a mixed stage cDNA library provided by A. Fire (personal communication). The DNA sequence was determined using the dideoxy chain terminator method (Sanger et al., 1977). Reverse transcription and polymerase chain reactions were used to detect *apx-1* mRNAs trans-spliced to SL1. The SL1 and SL2 primers and reaction conditions are described in Spieth et al. (1993). Genomic clones were tested for rescue as described in Mello et al. (1991).

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### GenBank Accession Number

The accession number for the sequence reported in this paper is U07628.

### Note Added in Proof

The data cited as S. Crittenden and J. Kimble, personal communication, will appear in the next issue of *Cell*.